university of groningen / faculty of science and engineering

The Pathogenesis of SARS-CoV-2

The life cycle of SARS-CoV-2 and the consequence for the human body

Master Thesis

Vivianne Veenma (S2993805)
Supervisor: prof. dr. A. L. W. Huckriede
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Abstract: *Being the third big outbreak of a coronavirus in the past two decades, SARS-CoV-2 has spread over the world and is claiming thousands of lives. To prevent more casualties and work towards a vaccine it is important to understand the pathogenesis and life cycle of the virus. The virus can spread in two ways; through respiratory droplets and the fecal-oral route. Both transmission routes result in the virus entering the lungs and attacking the alveoli cells. The virus attaches it's S spike to the ACE2 receptor of the host cell and enters either by way of an endosome or through direct membrane fusion. It then hijacks the host and replicates causing damage to the host cell, which results in the production of pro-inflammatory cytokines that attract immune cells. From here the patient can either experience a mild infection in which the patient will have symptoms like a fever, cough and difficult breathing, or a severe infection when the huge amount of cytokines produced (cytokine storm) migrate through the blood and cause Multi System Organ Failure (MSOF), which can be fatal. In the mild infection the cytokine storm is prevented by T cells and macrophages that clear the infection. Even though this review gives critical insight into the pathogenesis of SARS-CoV-2 there are remain a lot of gaps that need to be cleared by future research.*

1 Introduction

1.1 Corona viruses of the past

One of the major pathogens that primarily targets the human respiratory system is the Coronavirus (CoV) (Rothan, 2020). There have been several big outbreaks of this virus in the past. An example of this is Severe Acute Respiratory Syndrome (SARS)-CoV that emerged in 2002 in the Guangdong province in China. The virus spread to five continents through air travel routes, infecting over 8 000 people and causing 774 deaths (Dorsten, 2003; Ksiazek, 2003). In 2012 a new CoV named Middle East Respiratory Syndrome (MERS)-CoV emerged in the Arabian Peninsula and until date this virus remains a public health concern in this area (Lin, 2020). MERS-CoV spread to 27 countries infecting over 3 000 individuals and claiming 858 lives (Zaki, 2012).

The pathophysiology of the newly rising SARS-CoV-2 infection, resembles that of SARS- and MERS-CoV closely. It is characterized by aggressive inflammatory responses resulting in damage to the airways (Lu, 2020). The consequences to the body is therefore not only due to the viral infection but also the host's immune response to the virus. There is a huge individual difference between patients; some only experience minor symptoms resembling a common cold, while others get a severe immune response that can be fatal (Tay, 2020).

1.2 Epidemiology

In the city of Wuhan in Hubei province, the Chinese health authorities identified a cluster of pneumonia cases of unknown etiology in december 2019 (Lake, 2020). Features included acute respiratory distress, lymphopenia, fever and no response to antibiotic treatment. After spreading through the hospital and more cases were admitted, the patients were identified with laboratory confirmed COVID-19 infections (Morse, 2020). Links between the index cases and the city's South China Seafood Market were noted. At this market a large range of alive and freshly slaughtered animals were sold, including rats, bats and snakes. It is currently thought that the virus originates from bats, based on several analyses (Lu, 2020; Zhao, 2020). Others say that the bats were hibernating when the virus emerged and attribute it to pangolins (Zhang T. Q., 2020). The virus quickly spread across China and to other countries like Korea, Japan, Italy and Iran (Cao, 2020). From there it spread across Europe, America and the rest of the world, with a total of 4,12 mln cases and 283 120 deaths confirmed on the 11th of April 2020 (WHO, 2020).

1.3 Virology

Coronaviruses (CoVs) are enveloped viruses with a positive sense, single-stranded RNA genome, ranging from 26 to 32 kbs in length. They belong to the *coronaviridae* family in the Nivovirales order. These viruses present a crown-like spike on the outer surface of the virus, thus it was named coronavirus, Fig 1 (Shereen, 2020). These viruses have an extensive range of natural origin and can cause respiratory, hepatic, enteric and neurologic diseases (De Wilde, 2018; Weiss, 2011). The CoVs are divided into four subfamilies: α, β , γ and δ-CoVs, SARS and MERS-CoV are members of the β-CoVs (Weiss, 2011).

As shown in Fig 1, the SARS-CoV-2 virion, similar to other β-CoVs, has a genome size of 29.9 kb and possesses a nucleocapsid composed of genomic RNA and phosphorylated nucleocapsid (N) protein. The nucleocapsid is buried inside phospholipid bilayers and on the outside of the membrane the spike glycoprotein trimmer (S), which exist in all CoV, is present (Jin, 2020). The membrane (M) protein and the envelope (E) protein are located among the S proteins in the viral envelope (Weiss, 2011).

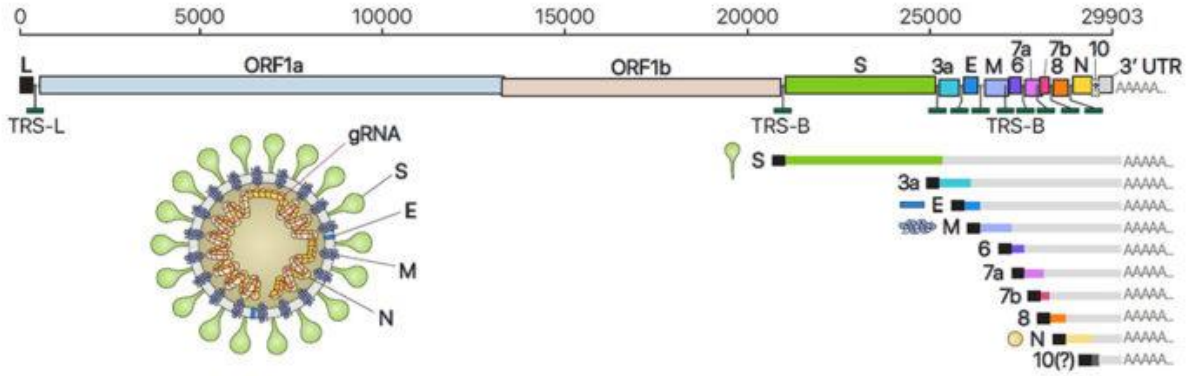

Figure 1: Coronavirus virion structure and single-stranded RNA genome. *The genomic RNA consists of 29 903 nucleotides that also serves as mRNA, from which ORF1a and ORF1b are translated. The positive single stranded RNA is enveloped by a viral membrane and the N protein (N) binds to the viral RNA. On the membrane (M) spikes (S) are visible and a small membrane envelope (E) is visible in the membrane* (Kim, 2020).

1.4 Research question and relevance

Even though joint prevention and quarantine mechanisms have been enacted, the virus is spreading and still possesses a serious threat to global public health (Gorbalenya, 2020). Due to a lack of specific antiviral treatments and pressure of clinical treatment, thousands of severe cases die every day worldwide (Jin, 2020).

In this review I will present and discuss the SARS-CoV-2 transmission, cell entry, replication and symptoms, this will give some much needed insight into the pathogenesis of the virus and might help towards a treatment and ultimately prevent more casualties. The main goal of this review is to answer the question; what happens in the body during a SARS-CoV-2 infection.

2 Routes of Transmission

SARS-CoV-2 is mainly transmitted by human-to-human contact for example by the inhalation or contact with infected droplets. Besides this there is also evidence for a fecal oral route (Singhal, 2020).

2.1 Respiratory droplets

Humans can produce respiratory droplets in several ways, including breathing, talking, sneezing, coughing or singing (Toth, 2004). Naturally produced droplets by humans consist mostly of water, but also include many cell types, like epithelial and immune cells, physiological electrolytes, like Na^+ and K^+, and various infectious agents (Atkinson, 2009). Droplets can be transmitted in different sizes. Drops bigger than 5 µm in diameter are referred to as respiratory droplets, these mainly get trapped in the upper respiratory tract and fall rapidly to the ground under gravity. Therefore respiratory droplets can only be transmitted over a limited distance (Stetzenbach, 2004). Droplets smaller than 5 µm are called droplet nuclei and these have the potential to be inhaled into lower respiratory tract; the bronchi and alveoli. In contrast to respiratory droplets, droplet nuclei can remain suspended in the air for significant periods of time, this allows them to be transmitted over distances greater than 1 meter (Atkinson, 2009; Burke, 2020).

The most common transmission routes of SARS-CoV-2 is direct transmission, including cough, sneeze and droplet inhalation, and contact transmission, contact with oral, nasal and eye mucous membranes (Liu X. L., 2020). According to current research, COVID-19 is primarily transmitted between people through respiratory droplets and contact routes (Burke, 2020).

Droplet transmission occurs when a person is in close contact, usually within 1 meter, with someone who has respiratory symptoms and is able to transfer potentially respiratory droplets including virions to exposed mucosae or conjunctiva of a healthy person (Zhang J. J., 2020). Infection may also be spread by fomites in the environment of an infected person. Therefore, transmission can occur by direct contact with an infected person and by indirect contact through infected surfaces in the environment or objects used by an infected person (WHO, 2020; Xiang, 2020).

2.2 Fecal oral route

The fecal-oral route is a route of transmission in which the virus in fecal particles pass from one person to another person's mouth. Main causes for this transmission include lack of adequate sanitation and poor hygiene (Davis, 2018).

New insights indicate that SARS-CoV-2 not only spreads through respiratory droplets or environmental contact, but also through the fecal-oral route. Several case studies have reported gastrointestinal symptoms and

evidence of viral RNA or live infectious virus present in faeces. *Xiao et al.* noted that the ACE2 protein, the receptor which leads to cell entry for the virus, is also expressed in the glandular cells of gastric, duodenal and rectal epithelia (Xiao, 2020). Additionally they noted that a significant number of patients experience diarrhea, vomiting, nausea, and abdominal discomfort and detected viral SARS-CoV-2 in the fecal samples. Correspondingly, *Gu et al.* noted enteric involvement and the presence of the virus in the intestines of patients with a COVID-19 infection (Gu, 2020). Likewise *Xu et al., Wang et al.,* and *Yun et al*. found evidence of the virus in the gastrointestinal tract and propose a fecal-oral route (Yun, 2020; Xu Y. L., 2020; Wang W. X., 2020).

However the exact mechanism of SARS-CoV-2 induced gastrointestinal symptoms largely remain elusive. It is thought the virus uses the ACE2 receptor, as suggested by both *Hindson et al.* and *Xiao et al.,* since evidence is found that ACE2 mRNA is highly expressed in the gastrointestinal tract (Hindson, 2020; Xiao, 2020).

3 Viral entry

Viral infections are dependent on the entry of the virus in cells so they can hijack the cellular machinery of the host cell, replicate and finally release huge amounts of viral copies (South, 2020). To successfully initiate an infection, viruses need to overcome the cell membrane barrier.

3.1 ACE2

It is known that SARS-CoV-2 virions use the host protein Angiotensin-Converting Enzyme-2 (ACE2) to gain entry into specific cells (Xu Y. L., 2020). ACE2 is richly present in human epithelia in the lungs and the small intestine but mRNA has also been found in other organs like the heart, arteries and kidney (Hamming, 2004; Gembrandt, 2005). Loss of ACE2 can lead to cardiomyopathy, pulmonary injury and kidney disease (Oudit, 2009).

ACE2 is a membrane-bound peptidase with the main function to lower blood pressure by catalyzing the hydrolysis of angiotensin II into angiotensin (1-7), which works as a vasodilator (Xu Y. L., 2020). It thus reduces the amount of angiotensin-II and increases Angiotensin (1-7) (Pasha, 2014; Burell, 2004). *Hashimoto et al.* identified a molecular crosstalk between the function of the ACE2 receptor and intestinal amino acid homeostasis, suggesting that there is a direct link between the intestinal microbiotica and the innate immunity that might be related to ACE2 (Hashimoto, 2020).

3.2 Entry in the cytoplasm

It is believed that SARS-CoV-2 can cross the cell membrane in two ways; via indirect entry via endosomes or through direct entry at the plasma membrane (Bosch, 2003; Pampel, 2020). As visible in Fig 1, the SARS-CoV-2 virion membrane expresses a protein termed spike glycoprotein trimmer (S). This spike contains a receptor binding region, in the S1 region, that binds, in both entry methods to the extracellular domain of ACE2 with high affinity (South, 2020).

When the virus enters via an endosome, the S1 region binds to the ACE2 receptor translocating the ACE2-virus complex to an endosome, a schematic representation is visible in Fig 2-1a. In the endosome an endosomal acid protease, called cathepsin L, activates the spike protein by cleaving the protein into S1 and S2 (Belouzard, 2009; Shereen, 2020). The S2 protein fuses the membrane of the virus with the membrane of the endosome leading to the release of the virus into the cytoplasm (Ou, 2020; Cong, 2020).

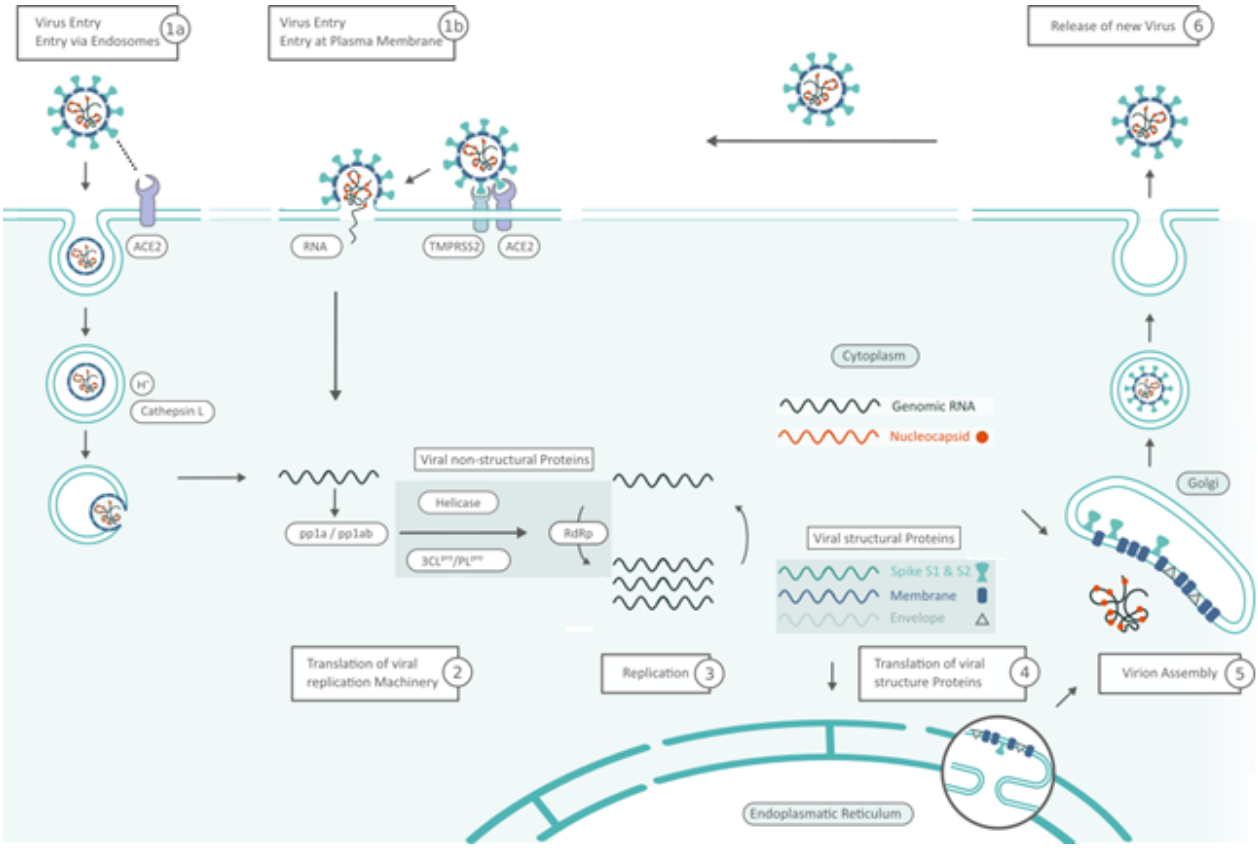

Figure 2: Life cycle of SARS-CoV-2. *The virus can enter via the ACE2 receptor either through an endosome, after which it can fuse with the membrane (1a) or via the plasma membrane (1b), both methods result in the release of the RNA in the cytoplasm of the host cell. From this RNA pp1a and pp1ab are translated and the RNA replicase transcription complex (RdRp) is formed through* $3CL^{PRO}$ *and* PI^{pro}*(2). The RdRp will drive the replication (3) and viral structural proteins are formed that will form the spike, membrane and envelope of the new virion particle (4). In the endoplasmic reticulum-Golgi apparatus the proteins will merge into a new virus particle together with the genomic RNA and the nucleocapsid (5) and these are transported out of the cell via exocytosis (6)* (Pampel, 2020).

Alternatively, the spike protein can bind to the ACE2 receptor and be activated by the Type II Transmembrane Serine Protease (TMPRSS2), which initiates fusion of the viral membrane with the plasma membrane (Hoffmann M. W., 2020; Zhu, 2013). The virus can directly release the RNA into the cytoplasm for replication, shown in Fig 2-1b (Rabi, 2020; Cong, 2020). The plasma membrane fusion entry is less likely to trigger host antiviral immunity since it doesn't change the amount of ACE2 receptors. In the endosomal pathway less ACE2 receptors are present on the cell surface because the ACE2 is translocated during viral entry, this is noted by immune cells which cause the host cells to go into apoptosis. Therefore the direct membrane fusion is more efficient for viral replication (Shirato, 2018; Wang Q. Z., 2020).

4 Coronavirus replication

Once the viral RNA is released into the host cell it hijacks the replication of the cell to form new virus particles that can infect other cells. This happens in four steps; the translation of viral replication machinery, replication of the genome, translation of viral structure proteins and finally, the virion assembly, a schematic summary is shown in Fig 2.

When the viral RNA is released into the host cell the viral genome is first unveiled in the

cytoplasm, see Fig 1, for a complete representation of the SARS-CoV-2 genome (Song, 2019). ORF1a and ORF1ab are translated to produce pp1a and pp1ab viral replicase polyproteins. The polyproteins are cleaved by the Papain like protease (PI^{pro-}) and 3C-like protease ($3CL^{PRO}$) which are encoded by ORF1a-b (Shereen, 2020). This results in 16 non-structural proteins; NSP1-11 are encoded in ORF1a and nsp12-16 are encoded in ORF1b. These replicase-transcriptase proteins, together with other viral proteins and, possibly, cellular proteins, assemble into the RNA replicase transcription complex (RdRp) (Sawicki, 2007). This complex drives the production of negative sense RNA through both replication and transcription (Du, 2009).

Negative sense RNA intermediates are generated to serve as the templates for the synthesis of positive sense genomic RNA (gRNA) and subgenomic RNA (sgRNA) (Kim, 2020). The RdRp uses the (+) strand gRNA as a template, which will become the genome of the new virus particle.

sgRNAs produced through the transcription are translated into structural proteins; spike proteins (S), envelope proteins (E), membrane proteins (M) and nucleocapsid proteins (N), together they form the new viral particles. Spike, envelope and membrane proteins enter the endoplasmic reticulum and the nucleocapsid protein is combined with the (+) strand genomic RNA to become a nucleoprotein complex. In the endoplasmic-Golgi apparatus complex, the proteins merge into a complete virus particle, and are excreted from primary cells to extracellular regions through the Golgi apparatus via exocytosis (Kim, 2020). The mature virions can infect new target cells which results in the production of more virus particles (Song, 2019).

5 Cytokine storm

Once the virus has managed to enter and replicate in the host, Fig 3-1, the immune system responds.

5.1 Early onset of the infection

The early onset of rapid viral replication may cause epithelial and endothelial cell pyroptosis, which is a programmed cell death dependent on caspase-1 (Bergsbaken, 2010). Pyroptosis causes the release of Damage Associated Molecular Patterns (DAMPs); e.g. nucleic acids and ATP (Yang M. , 2020). DAMPs are recognized by neighboring endothelial-, epithelial-cells and alveolar macrophages, triggering the formation and release of pro-inflammatory cytokines and chemokines; IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1 (Pedersen, 2020). These pro-inflammatory cytokines and chemokines attract monocytes, macrophages and T cells to the site of the infection (Zhang C. W., 2020). These cells promote further inflammation establishing a pro-inflammatory feedback loop, shown in Fig 3-2 (Tay, 2020).

5.2 Healthy immune response

In a healthy immune response, Fig 3 bottom right corner, the initial inflammation attracts virus-specific T cells to the site of infection, which have been recruited by dendritic cells in the lymphe node (Li, 2020). These eliminate the infected cells before the virus spreads. Neutralizing antibodies in these individuals can block viral infection. Alveolar macrophages recognize neutralized viruses and apoptotic cells and clear them by phagocytosis (Shi, 2020). These processes lead to clearance of the virus and minimal lung damage, ultimately resulting in recovery (Tay, 2020).

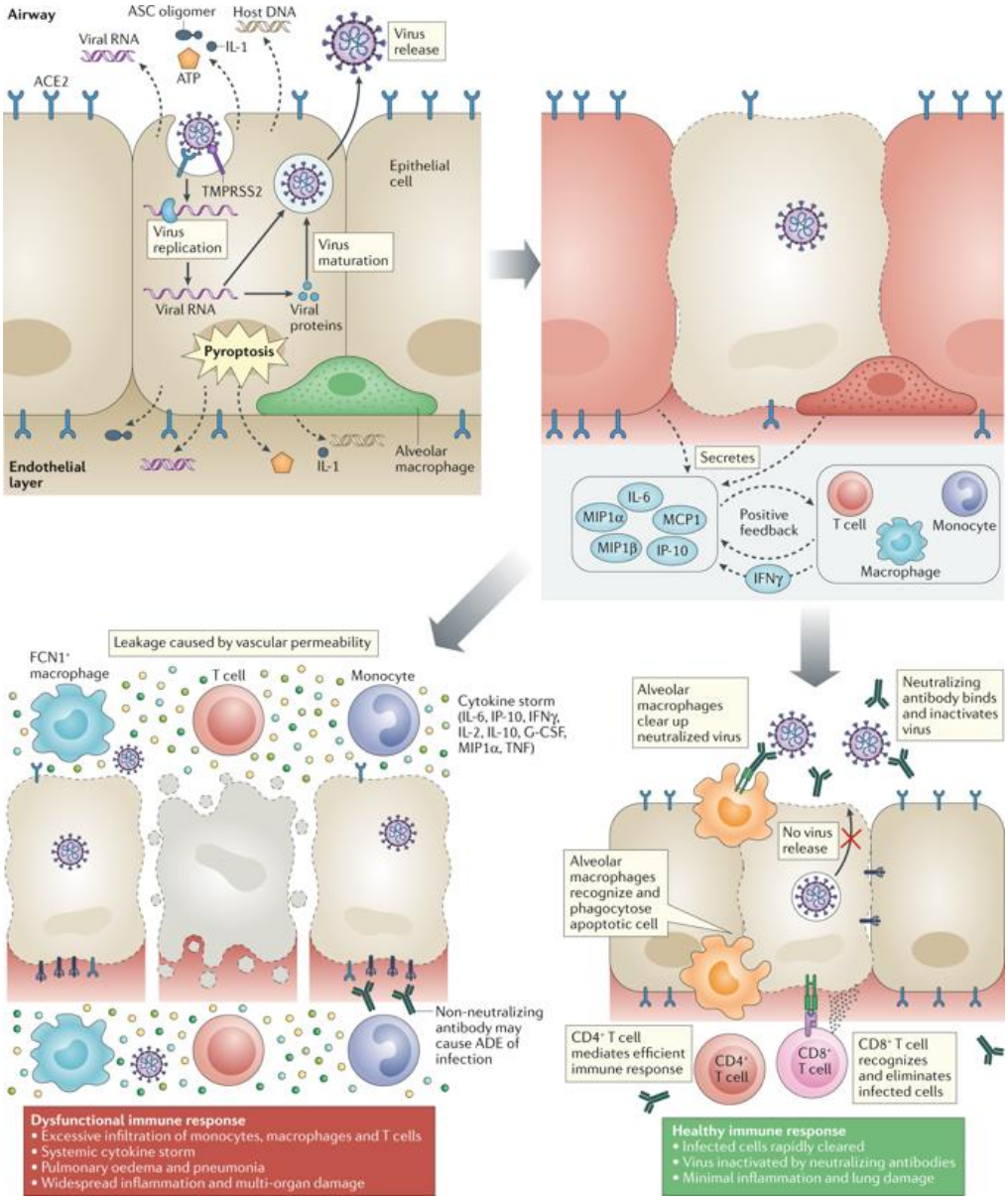

Figure 3: Chronological events of a SARS-CoV-19 infection*. The virus enters the cell and hijacks the host's replication system, the virus is replicated and released to infect other cells. In the progress the cell is damaged and undergoes* pyroptosis*, DAMPs are released and recognized by neighboring cells that excrete IL-6, MCP1, IP-10, MIP1ϐ and MIP1α, which attract T cells, monocytes and macrophages, which will also produce these pro-inflammatory cytokines and chemokines and cause a positive feedback loop. From there it can go two ways. In the dysfunctional immune response the immune cells will start to accumulate and damage the lung infrastructure, furthermore the inflammation can spread through the body. In the healthy immune response the virus will be cleared through T cells and macrophages* (Tay, 2020).

5.3 Dysfunctional immune response

In a dysfunctional immune response, Fig 3 bottom left corner, the early onset leads to further accumulation of immune cells in the lungs causing an overproduction of pro-inflammatory cytokines, which damages the lung infrastructure (Li, 2020; Zhang C. W., 2020). On top of this neutrophils are attracted by cytokines and migrate from the blood to the infection site, these will try and destroy the virus by releasing Reactive Oxygen Species (ROS) and proteases (Shi, 2020). Even though this destroys the virus it also damages the surrounding tissue, causing an even more severe inflammation. Due to this uncontrolled inflammation a huge amount of cytokines are produced causing a

cytokine storm. The cytokine storm may circulate via the blood to other organs, leading to multi organ damage. In addition, B cells are attracted and produce non-neutralizing antibodies to neutralize the virus but this causes enhancement of the SARS-CoV-2 infection through antibody-dependent enhancement (ADE) (Tay, 2020). More of the severe immune responses affecting the whole body can be read in chapter 6 (Pedersen, 2020).

6 Defining the symptoms of COVID-19

In most individuals with a SARS-CoV-2 infection, immune cells are recruited and the infection in the lung recedes resulting in recovery (Tay, 2020). Symptoms are usually minor and limited to a cough, difficulty breathing and fever, resembling those of a common cold (Lin, 2020). However in some individuals, as explained before, a dysfunctional immune response occurs, which triggers a cytokine storm that mediates widespread lung inflammation. These cytokines can circulate via the blood to other organs and lead to multi-organ damage, typically in the cardiac, hepatic and renal systems (Tay, 2020). This chapter was mainly based on information of SARS-CoV-2 but some gasps were filled up with knowledge from related viruses, like MERS-CoV-2, SARS-CoV or influenza. When this is the case the referenced article is published before 2020.

6.1 Symptoms of a Mild SARS-CoV-2 infection

Main symptoms in an individual with a mild infection (Fig 4) include a cough, difficulty breathing and a fever, these are mainly caused by the immune response to the virus (Lin, 2020). When the virus replicates in a host cell, DAMPs are recognized by the immune cells and IL-1, IL-6, TNF-α are excreted (Pedersen, 2020; Liu J. L., 2020). These cytokines migrate to the blood and cause dilation in the endothelial cells, increasing the capillary permeability and causing vasodilation (Doremalen, 2020; Zhang C. W., 2020). Due to this, plasma leaks out into the interstitial spacious and in the alveoli, which causes pressure on the alveoli (Zhang J. J., 2020). A compound called surfactant that is involved in the reduction of the surface tension in the lung will be migrating out of the alveoli (Anzueto, 2002). This causes the surface tension to go up, causing the alveoli to collapse. Alveoli collapse leads to decreased gas exchange and increased work of breathing, resulting in difficulty breathing (Sims, 2008; Monteil, 2020).

On top of this, when the cells in the alveoli are destroyed, the death cells start to accumulate in the middle of the alveoli together with fluid, protein deposition, macrophages and neutrophils (Shi, 2020). This likewise alters the gas exchange leading to hypoxemia and collapse of the alveoli. The patient will also start coughing up this accumulation of debri when it starts to degrade, causing the cough (Pedersen, 2020).

The fever is caused when cytokines, e.g. IL-6, produced at the site of infection, travel through the blood to the Central Nervous System (CNS) (Pedersen, 2020; Schmitz, 2005). The hypothalamus senses high concentrations of cytokines, and releases prostaglandins, like PGE2, to increase the body temperature causing a fever (Conti, 2016).

Finally, because there is lower gas exchange possible due to collapsing alveoli and the buildup of fluid and debri, there will be a lower concentration of oxygen in the blood (PO2), which stimulates the chemoreceptors. This will trigger a reflex and stimulate the Sympathetic Nervous System (SNC) to increase the patient's heart rate to make up for the low PO2 and get enough oxygen to the vital organs (Shi, 2020; Tay, 2020).

Nevertheless in patients with a mild infection the infection will be cleared preventing a cytokine storm, and no severe lung damage is induced.

6.2 Symptoms of a Severe SARS-CoV-2 infection

In patients with a severe SARS-CoV-2 infection (Fig 4) the before mentioned course of events also take place and the individual will experience similar symptoms like a cough, fever and difficult breathing, at the beginning of the infection. However, because the infection cannot be cleared by the T cells and macrophages, the infection in the lungs becomes so severe that cytokines will spread through the body (Pedersen, 2020). This will lead to Systemic Inflammatory Response Syndrome (SIRS) which is an exaggerated defense response of the body in response to the virus (Zhang C. W., 2020).

SIRS includes the release of acute phase reactants which are direct mediators of widespread autonomic, hematological, immunological and endocrine alterations in the subject (Morse, 2020).

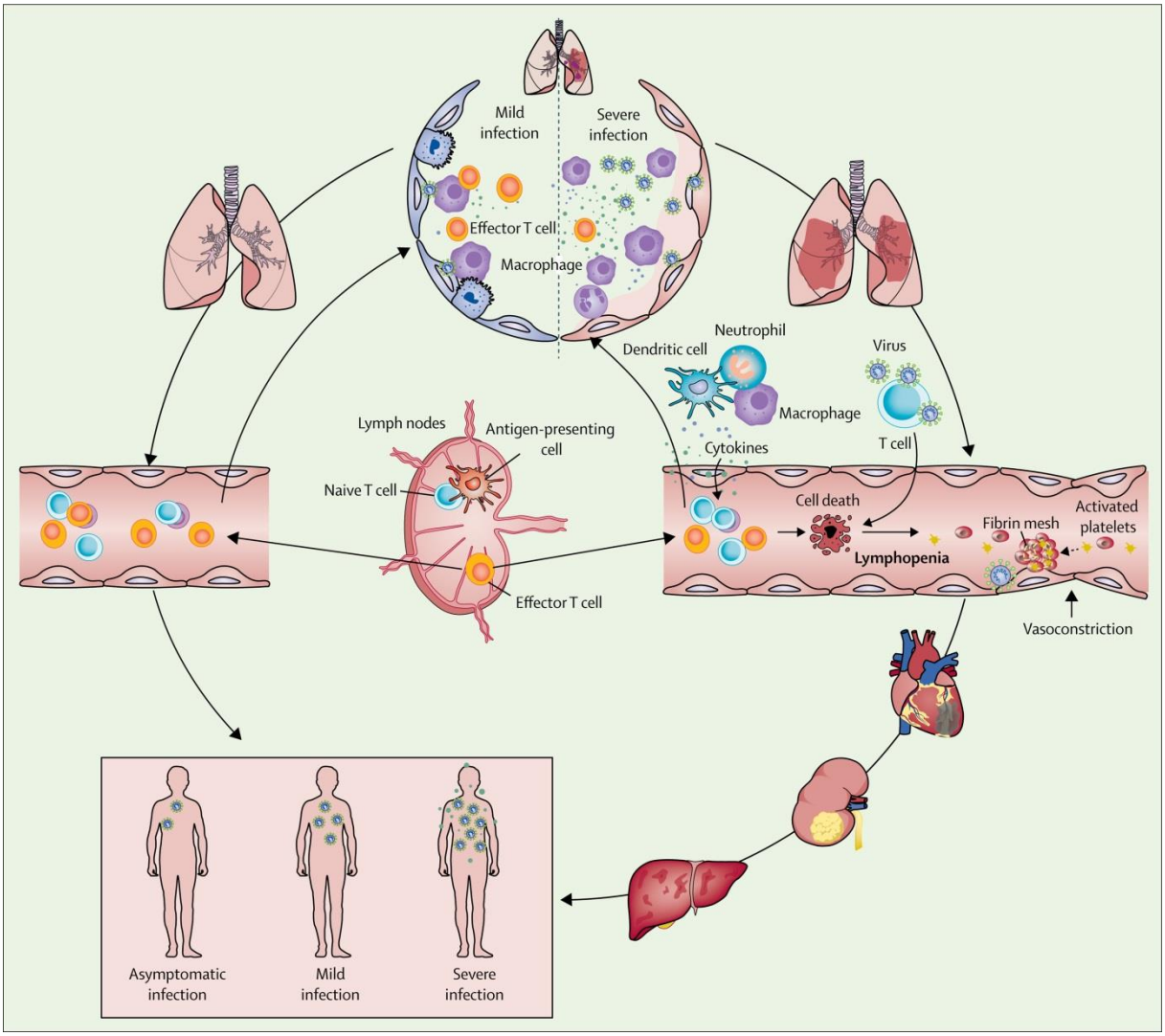

Figure 4: Pathogenesis of SARS-CoV-2; mild vs severe infection*: On the top an alveolus is visible in which the virus is attacking the cells and the immune cells try to defend the body. In a mild infection the T cells are recruited by dendritic cells and these will go to the location of the infection and with macrophages clear the infection. When a severe infection occurs the infection will not be cleared by the macrophages and T cells causing a cytokine storm and this can spread through the body and affect multiple organs* (Li, 2020).

Even though SIRS is meant to be defensive; to localize and eliminate the source, the deregulated cytokine storm can lead to a massive inflammatory cascade ultimately leading to organ dysfunction and even death (Chakroborty, 2020). When the inflammation spreads through the circulatory system this will cause increased capillary permeability within the systemic circulation. Fluid will start to leak out and accumulate into tissue spaces causing the blood volume to decrease. On top of this the infection will lead to vasodilation; the peripheral resistance will drop. Both the decrease in blood volume and drop in peripheral resistance will cause the blood pressure to drop, resulting in hypotension (Liu P. P., 2020). Hypotension will decrease the perfusion to multiple organs which can lead to Multi System Organ Failure (MSOF) (Huang, 2020; Wong, 2004).

Another danger is the immune suppression stage that is followed by the pro inflammatory phase. It is characterized by a reduction in the peripheral lymphocyte counts and is associated with a high risk of developing a bacterial infection. This condition known as lymphopenia has been found in several SARS-CoV-2 patients (Zhou, 2020; Li, 2020). Consequently both the immune suppression stage and MSOF can be fatal to the patient.

7 Discussion and Conclusion

This review contained a thorough description of the pathogenesis of SARS-CoV-19. It was proposed how the virus is transmitted, hijacks the cell and what happens in the body during a severe and mild virus infection. However, since SARS-CoV-19 is a rather new virus the pathogenesis described above is still debatable and full of gaps I will further discuss in this chapter.

7.1 Individual differences

All things considered, the main question regarding the pathogenesis of SARS-CoV-2 still remains; what is the underlying mechanism that defines whether an individual gets a dysfunctional or healthy immune response to this virus. Even though no conclusive results have been published to answer previous query, some researches have made suggestions for the underlying mechanism. The most likely mechanism seems to be the individual differences in the immune response, *Liu et al.* suggest that the loss of T cells during the SARS-CoV-2 infection may result in aggravated inflammatory responses, while in other individuals the T cell numbers are restored and elevated. In line with this hypothesis they observed that the kinetic changes of T cells counts correlated with the kinetic changes of the cytokine levels in the peripheral blood in COVID-19 patients (Liu J. L., 2020).

Other articles make suggestions related to the link between the age of the individual and the severity of the infection, which seems to become more severe the older a person is (Rothan, 2020). *Franklin et al.* suggest that this might be due to the vaccination against measles, mumps and rubella (MMR), a vaccine given to children since the 1970s (Fanklin, 2020). They identified 29% amino acid sequence homology of the macro domains between SARS-CoV and the rubella virus and a homology between the fusion proteins of SARS-CoV-2 and the measles and mumps viruses. Therefore they propose that the MMR vaccination might cause protection against SARS-CoV-2 and might prevent a severe infection (Fanklin, 2020). This theory is correspondingly suggested by *Gold et al.* (Gold, 2020) as well as *Stebbing et al.* who proposed the MMR vaccination as a possible treatment (Stebbing, 2020). This might be a possibility and play a small part but I

personally don't find this likely because no evidence has been found in case studies of this.

Another possibility for the individual differences might be that the likelihood of having a chronic condition increases in elderly. Individuals with a chronic condition, like cardiovascular problems are more susceptible to both SARS-CoV and SARS-CoV-2 (Wu, 2004; Yang J. Z., 2020). Nevertheless a combination of all these factors might be into play regarding the individual differences but I think it most likely that there is a difference in the immune response based on the study by *Liu et al.*

7.2 Is the fecal-oral route real?

In this rapport I highlighted the possibility of a fecal oral route of SARS-CoV-2. However, most articles describe the transmission route of SARS-CoV-2 solitary through respiratory droplets, e.g. *Peng et al.,* and *Liu et al.* On top of this, there has been till date limited research about the mechanism of action in the fecal oral route in this specific virus (Peng, 2020; Liu T. H., 2020). Nevertheless evidence of mRNA of the virus has been found in the stool samples of patients with a SARS-CoV-2 infection by multiple different research groups; *Xiao et al., Gu et al., Yu et al., Xu et al.* and *Wang et al.* (Xiao, 2020; Gu, 2020; Yun, 2020; Xu Y. L., 2020; Wang W. X., 2020). Moreover, it is suggested that the closely related virus MERS-CoV, is also able to spread through fecal oral transmission (Goh, 2013). Regarding the above named evidence, the fact that the ACE2 receptor is also found by *Hamming et al.,* and *Gembrandt et al.,* on enterocytes and that *Hashimoto et al.* and *Lamers et al.,* reported that the ACE2 can influence the composition of the gut microbiotica, I think it highly plausible that the fecal-oral transmission is real in SARS-CoV-2 and might have had a role in the spreading of the virus in specific circumstances (Hamming, 2004; Gembrandt, 2005; Hashimoto, 2020; Lamers, 2020). One of these settings was the Diamond Princess cruise ship with 3 700 people among which at least a third have been confirmed with a SARS-COV-2 infection possibly due to transmission via sewage, waste and contaminated water (Yuen, 2020; Rhiou, 2020).

7.3 Direct membrane fusion, endosomal pathway or both?

Another point of debate remains the virus entry, which is in some articles described as limited to the endosomal pathway, e.g. by *Du et al, Shereen et al.,* and *Song et al.* (Song, 2019; Du, 2009; Shereen, 2020). Nevertheless these articles are not as detailed and don't describe other important details like how the virus might make it out of the endosome. On top of this, it isn't accurate how these researchers describe the entry through the endosomal pathway using the TMPRSS2 protein (Ward, 2020). New research by *Tay et al*. show evidence of both an endosomal pathway and direct membrane fusion (Tay, 2020). *Shirato et al*. support this claim; they inhibited the endosomal pathway and found that the virus could still enter the host cell through TMPRSS2 (Shirato, 2018). Similarly Hoffman et al. concluded that the host cell could use both the endosmal pathway and the direct membrane fusion and investigated the TMPRSS2 protein in more detail (Hoffmann M. W., 2020). Additionally, the closely related SARS-CoV can also fuse directly with the cell membrane to release the RNA into the cytoplasm (Inoue, 2007; Wang H. Y., 2008). This evidence makes it likely that both these pathways can be used by SARS-CoV-2, nonetheless more research is needed to be more conclusive about this.

7.4 Unidentified underlying pathways

Due to the limited research regarding the specific virus replication and cytokine storm I guess there might be more processes involved in the specific immune response to the virus. Moreover, when referencing clinical features a lot of articles mention long lists of cytokines and chemokines that does not come into play in proposed pathogeneses. An example of such a research is the report by *Huang et al.* which mentions finding the following cytokines and chemokines in the blood plasma of patients with a SARS-CoV-2 infection; IL1B, IL1RA, IL2, IL4, IL5, IL6, IL7, IL8), IL9, IL10, IL12p70, IL13, IL15, IL17A, Eotaxin, basic FGF2, GCSF (CSF3), GMCSF (CSF2), IFNγ, IP10 (CXCL10), MCP1 (CCL2), MIP1A (CCL3), MIP1B (CCL4), PDGFB, RANTES (CCL5) (Huang, 2020). More research is necessary to understand how and whether these chemicals come into play in the immune response to SARS-CoV-2 and this might give an answer to the still open questions regarding the pathogenesis.

7.5 Recent hot topic in research, a blessing or a curse?

The biggest debatable issue of this review remains that most of the referenced articles are very recent due to the fact that SARS-CoV has only emerged very recently (December 2019). Which means that though SARS-CoV-2 is a hot item in research, most data and reports are still limited and not confirmed by other research organizations. Another problem is that most reports are preliminary and have not been peer reviewed, nevertheless they have been critically reviewed and were only used if they were accurate for this rapport.

There is limited knowledge about the details of the virus replication and the cytokine storm because there aren't as much published in debt about these topics. This is probably due to the fact that designing a research model regarding cells or animal models takes a lot of time. Though multiple reviews and researches do mention the cytokine storm and its consequences they don't describe the pathway. Therefore some data was extracted from closely related viruses, like SARS-, MERS CoV and influenza virus and is not yet validated on SARS-CoV-2, this has been noted in the text with references used published before 2020.

7.6 Conclusion

In sum, this research provided key insights into SARS-CoV-2 transmission, viral entry into cells, the immune response against the virus and the symptoms. Even though some gaps and uncertainties arise in certain points of this pathogenesis, the above described is the most likely for SARS-CoV-2. It is clear more research still needs to be conducted to get a more detailed insight in the pathogenesis, e.g. to understand why certain individuals get a severe or mild infection, or if the virus is indeed able to use direct membrane fusion to enter the host cells.

Several large scale immunity studies are necessary to provide insight into individual differences. On top of this, more virus-cell studies are essential to study the cell entry and in debt immune response, I suggest using organoids since these resemble a human alveolus in detail. It is also an ideal method to look into the immune response since organoids also contain macrophages and epithelial cells.

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